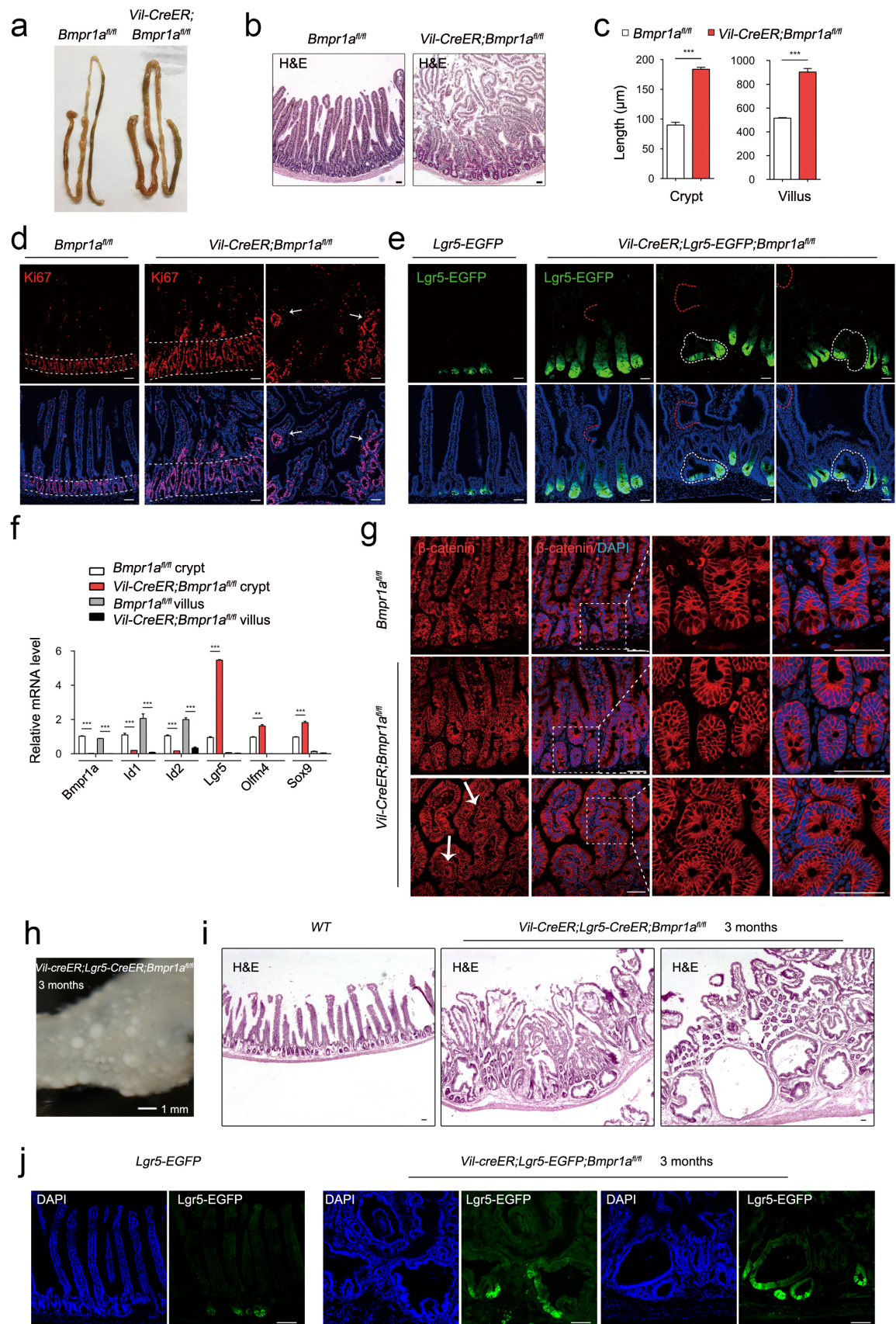


Supplementary Figure 1 BMP restricts Lgr5⁺ ISC self-renewal during homeostasis through a Wnt/ β -catenin-independent pathway.

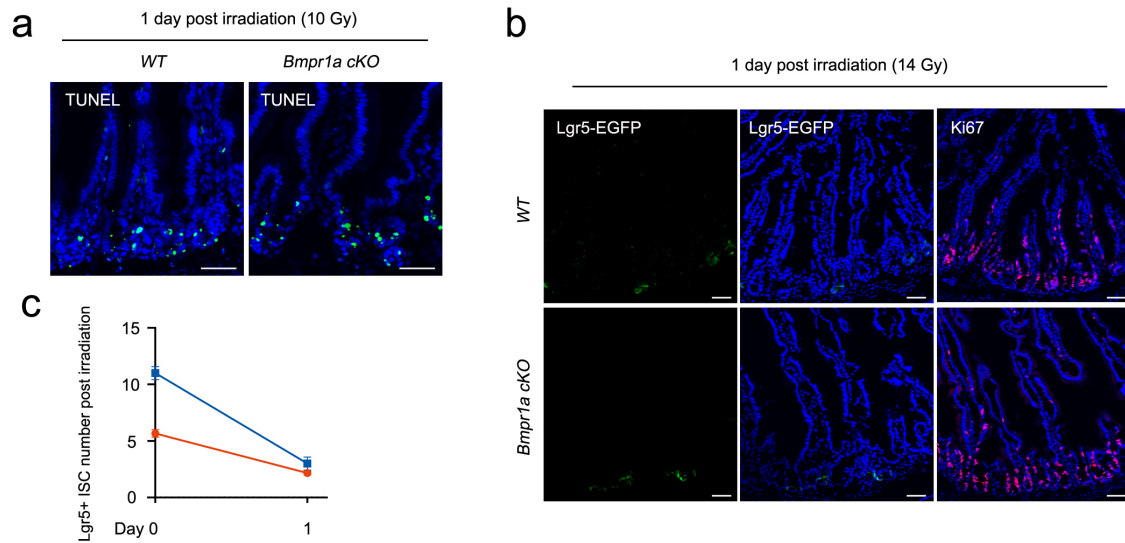
(a) Quantitative genomic PCR of crypt or villus DNA from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice at day 12 after the first injection of tamoxifen. Data represent mean \pm s.e.m of n=6 mice. *** $P < 0.001$ by Student's *t*-tests. (b) Quantitative RT-PCR of *Bmpr1a* expression in crypt or villus from the mice with indicated genotypes at day 12. Data represent mean \pm s.e.m of n=6 mice. *** $P < 0.001$ by Student's *t*-tests. (c) Immunoblots of small intestine epithelium from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice

at day 12. Data are representative of three independent experiments. (d) Body weight curve of *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice before analysis. (e) Macroscopic images of intestines from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice at day 12 and quantification of the intestine length and width as indicated. Images are representative of n=6 mice per genotype. Data represent mean \pm s.e.m of n=6 mice per genotype. *** $P < 0.001$ by Student's *t*-tests. (f) Immunofluorescence staining of Lysozyme in *Vil-CreER;Bmpr1a^{fl/fl}* and *Bmpr1a^{fl/fl}* mice at day 12. Nuclei were counter-stained with DAPI. Images are representative of n=6 mice per genotype. (g) Immunofluorescence staining for BrdU incorporation 2, 12 or 24 h after a single BrdU pulse in *Vil-CreER;Bmpr1a^{fl/fl}* and *Bmpr1a^{fl/fl}* mice. BrdU was injected at day 12. Images are representative of n=5 mice per genotype. (h) β -catenin and Ki67 immunofluorescence staining in transformed crypts compared with neighboring normal ones at a representative dysplastic region in intestine from 5 month-old *Vil-Cre;Apc^{wt/fl}* mice. The lower panels show the enlargements of boxed area. T stands for tumor, and N stands for normal tissue. Nuclei were counter-stained with DAPI. Images are representative of n=4 mice per genotype. Scale bars, 50 μ m.



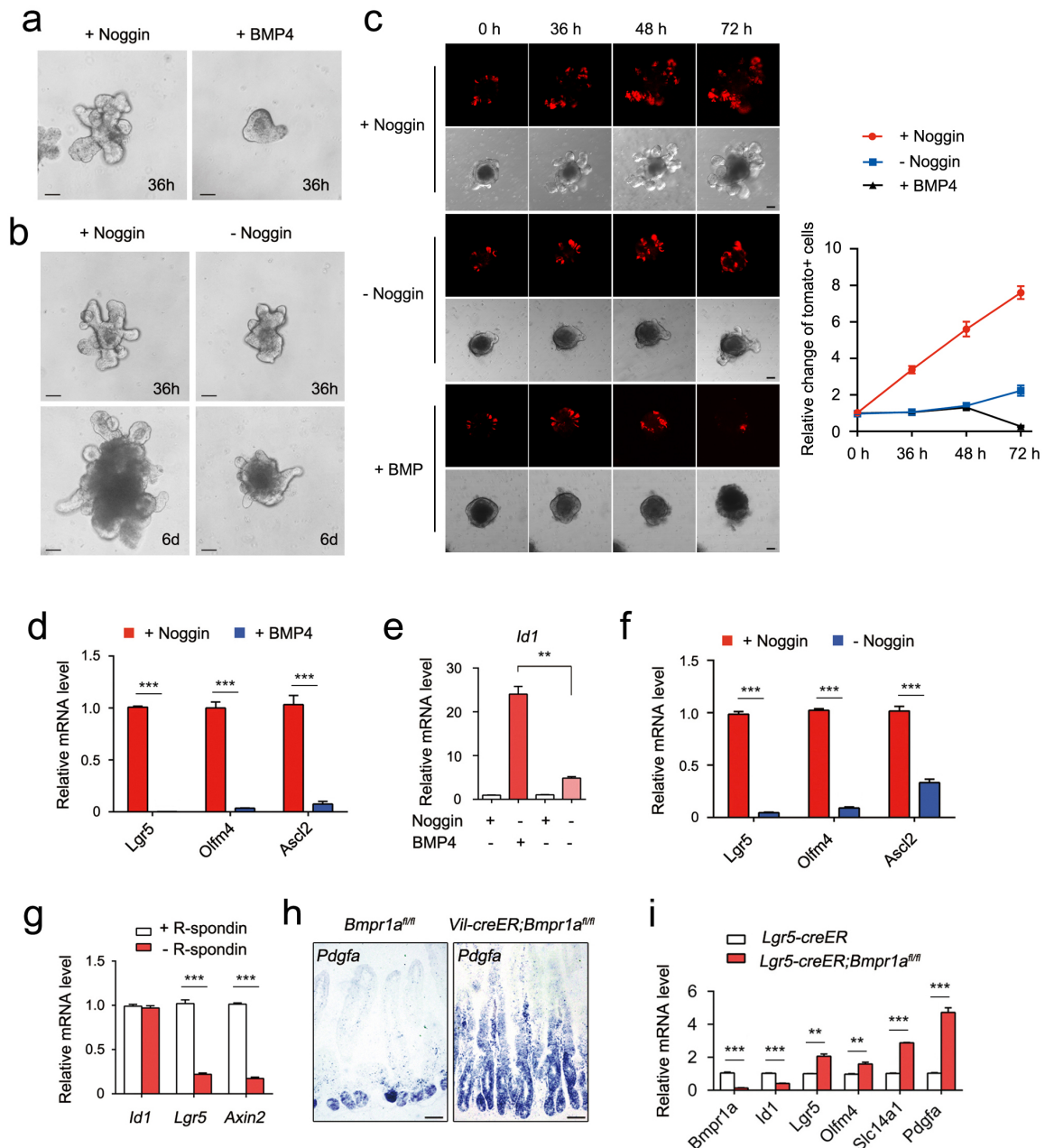
Supplementary Figure 2 Loss of BMP in intestinal epithelium drives intestinal dysplasia and polyp formation.

(a) Macroscopic images of intestines from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice at 1.5 months after 5-day tamoxifen administration. Images are representative of n=5 mice per genotype. (b,c) H&E staining of proximal jejunum sections and quantification of crypt length and villus length in *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice. Images are representative of n=5 mice per genotype. Data represent mean \pm s.e.m of n=5 mice per genotype. *** $P < 0.001$ by Student's *t*-tests. (d) Proximal jejunum sections were stained with Ki67. Dotted lines demarcate crypt region. Arrows mark crypt-like epithelium invaginations containing Ki67+ cells on the flanks of villus. Nuclei were counter-stained with DAPI. Images are representative of n=5 mice per genotype. (e) Lgr5-EGFP fluorescence of proximal jejunum sections in *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice. Dotted red lines demarcate villus invaginations, while dotted white lines indicate the microadenoma-like structures. Nuclei were counter-stained with DAPI. Images are representative of n=5 mice per genotype. (f) Quantitative RT-PCR analysis of *Bmpr1a*, *Id1*, *Id2*, *Lgr5*, *Olfm4* and *Sox9* expression in crypt or villus from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice. Data represent mean \pm s.e.m of three independent experiments. ** $P < 0.01$, *** $P < 0.001$ by Student's *t*-tests. (g) β -catenin immunofluorescence staining in intestine from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice. Arrows mark crypt-like epithelium invaginations. Nuclei were counter-stained with DAPI. Images are representative of n=5 mice per genotype. (h) Polyps in duodenum from *Vil-CreER;Lgr5-CreER;Bmpr1a^{fl/fl}* mice 3 months after 5-day tamoxifen injections. (i) H&E staining of polyps in the mutant mice. (j) Representative images of the Lgr5-EGFP⁺ stem cells at the base of polyps. Scale bars, 100 μ m. Scale bars, 50 μ m.



Supplementary Figure 3 Lgr5⁺ stem cells in *Bmpr1a cKO* mice do not show radioresistance.

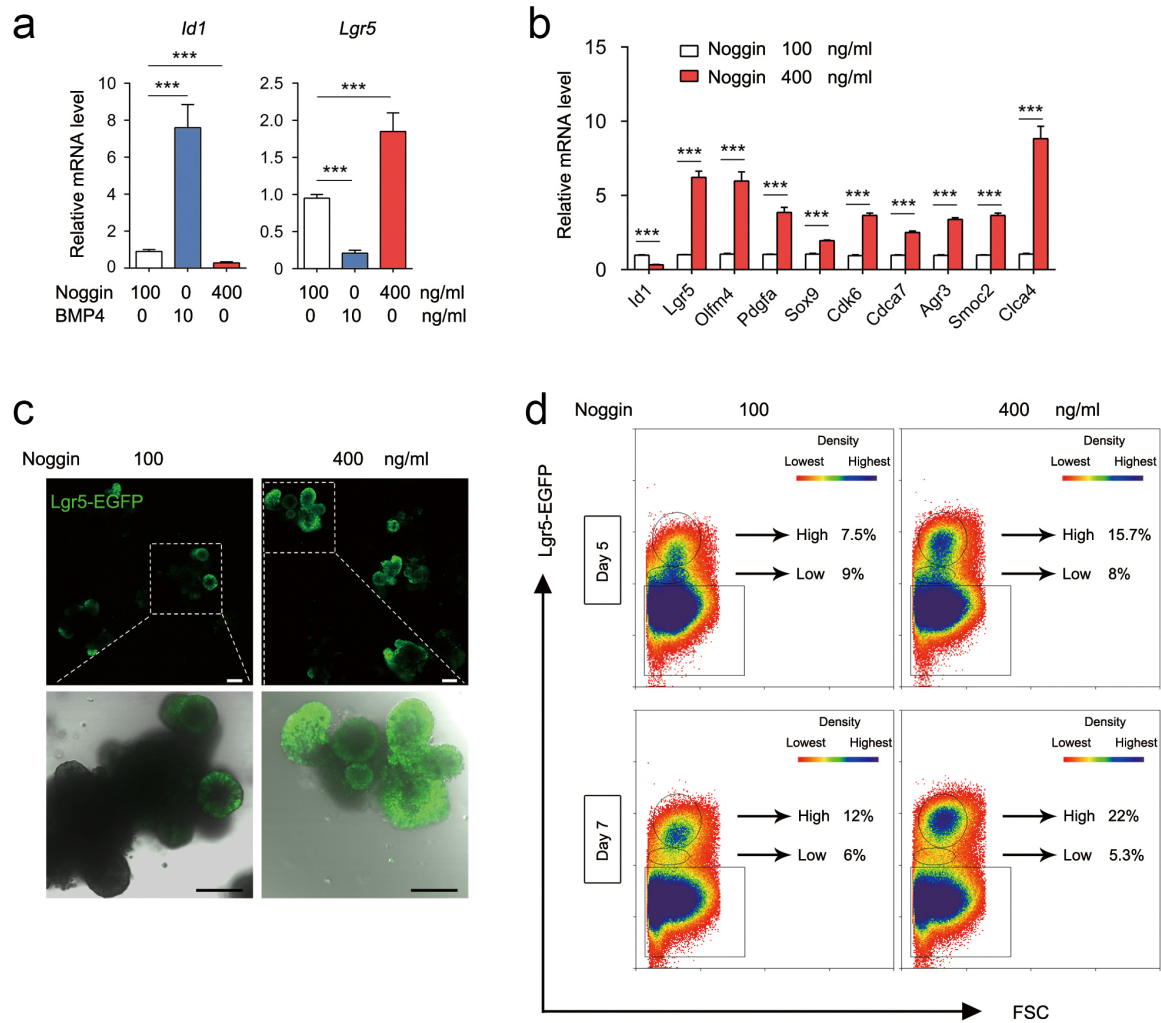
(a) Representative images of TUNEL assays in jejunum sections from *Bmpr1a^{fl/fl}* and *Vil-CreER;Bmpr1a^{fl/fl}* mice at day 1 after 10 Gy abdominal X-ray radiation (n=5 mice per genotype). (b) Representative images of Lgr5-EGFP immunofluorescence and Ki67 staining in jejunum sections from *Bmpr1a^{fl/fl}* and *Vil-CreER;Bmpr1a^{fl/fl}* mice at different time points after 14 Gy abdominal X-ray radiation (n=5 mice per genotype). The mice were irradiated two weeks following 5-day tamoxifen induction. (c) Quantification of Lgr5⁺ stem cell number in *Bmpr1a^{fl/fl}* and *Vil-CreER;Bmpr1a^{fl/fl}* mice at different time points after 14 Gy abdominal X-ray radiation. Data represent mean ± standard error of the mean (s.e.m.) of n=5 mice per genotype. Scale bars, 50 μm.



Supplementary Figure 4 BMP directly represses proliferation and expression of known stem cell markers of intestinal organoids *in vitro*.

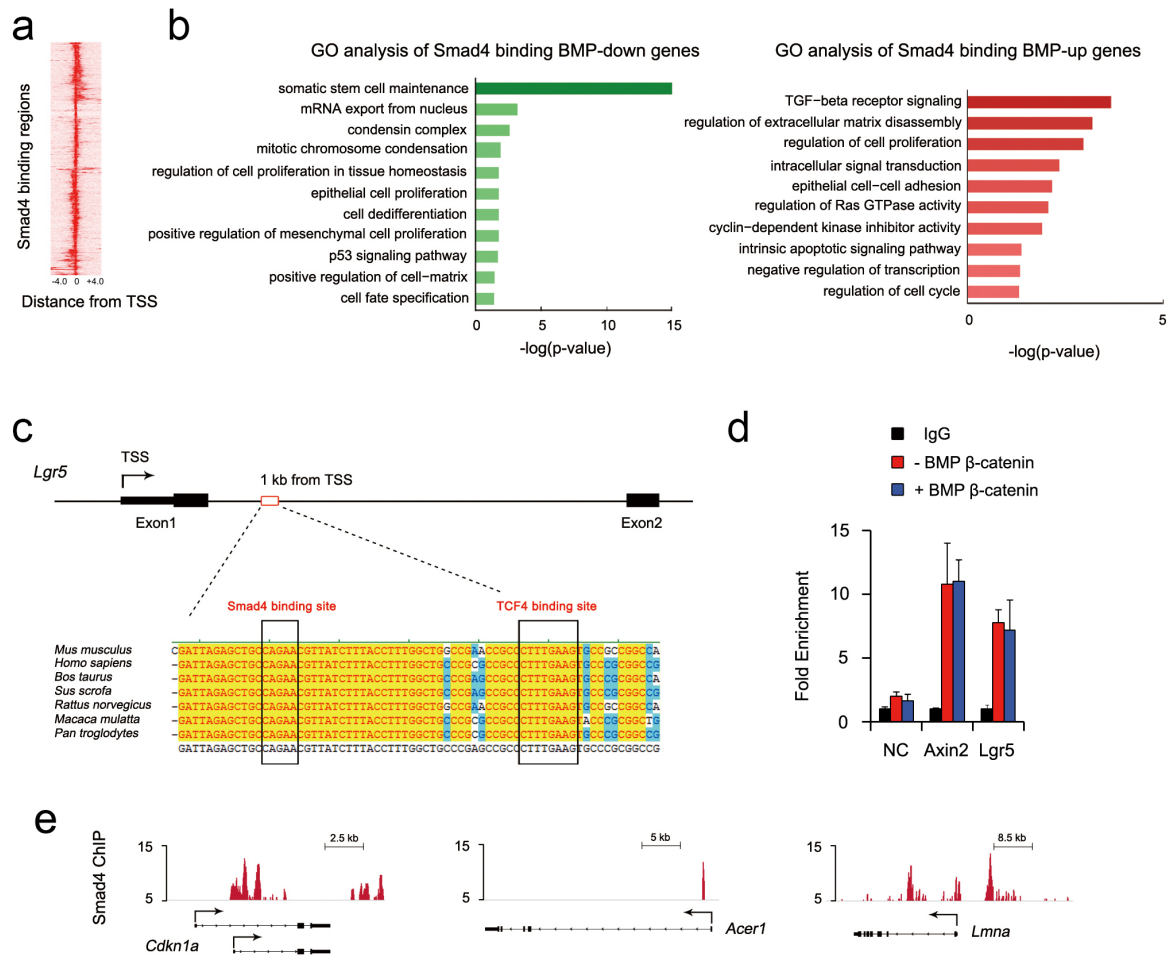
(a) The intestinal crypts were cultured to form organoids for 2.5 days in the ENR (EGF, Noggin and R-spondin) medium followed by direct BMP4 treatment for 36 hours. Images are representative of three independent experiments. (b) The intestinal crypts were cultured to form organoids for 2.5 days in the ENR (EGF, Noggin and R-spondin) medium followed by passaging. Images are representative of three independent experiments. (c) Lineage tracing of *Lgr5*⁺ stem cells in organoids derived from *Lgr5-CreERT2;td-Tomato* mice in indicated groups. 2-day old organoids were stimulated by 4-OHT for 12 hours followed by passaging. Representative images of tomato immunofluorescence in each groups at different time points were shown (n=3 independent experiments).

Quantification of tomato+ cells were shown aside. (d) Quantitative RT-PCR analysis of *Lgr5*, *Olfm4* and *Ascl2* in intestinal organoids after BMP stimulation for 24 hours. Data represent mean \pm s.e.m of three independent experiments. *** $P < 0.001$ by Student's *t*-tests. (e) Quantitative RT-PCR analysis of *Id1* in intestinal organoids after BMP stimulation or Noggin withdrawal for 4 hours. Data represent mean \pm s.e.m of three independent experiments. ** $P < 0.01$ by Student's *t*-tests. (f) Quantitative RT-PCR analysis of *Lgr5*, *Olfm4* and *Ascl2* in intestinal organoids after Noggin withdrawal for 24 hours. Data represent mean \pm s.e.m of three independent experiments. *** $P < 0.001$ by Student's *t*-tests. (g) Quantitative RT-PCR analysis of *Id1*, *Lgr5* and *Axin2* expression in organoids cultured in ENR medium or EN medium for 4 hours after passaging. Data represent mean \pm s.e.m of three independent experiments. *** $P < 0.001$ by Student's *t*-tests. (h) In situ hybridization of *Pdgfa* in intestines from *Bmpr1a^{fl/fl}* and *Vil-creER;Bmpr1a^{fl/fl}* mice at day 12. Images are representative of three independent experiments. (i) Quantitative RT-PCR analysis of *Bmpr1a*, *Id1*, *Lgr5*, *Olfm4*, *Slc14a1* and *Pdgfa* expression in Lgr5⁺ stem cells sorted from mice with indicated genotypes at day 12. Data represent mean \pm s.e.m of n=4 mice per genotype. ** $P < 0.01$, *** $P < 0.001$ by Student's *t*-tests. Scale bar, 50 μ m.



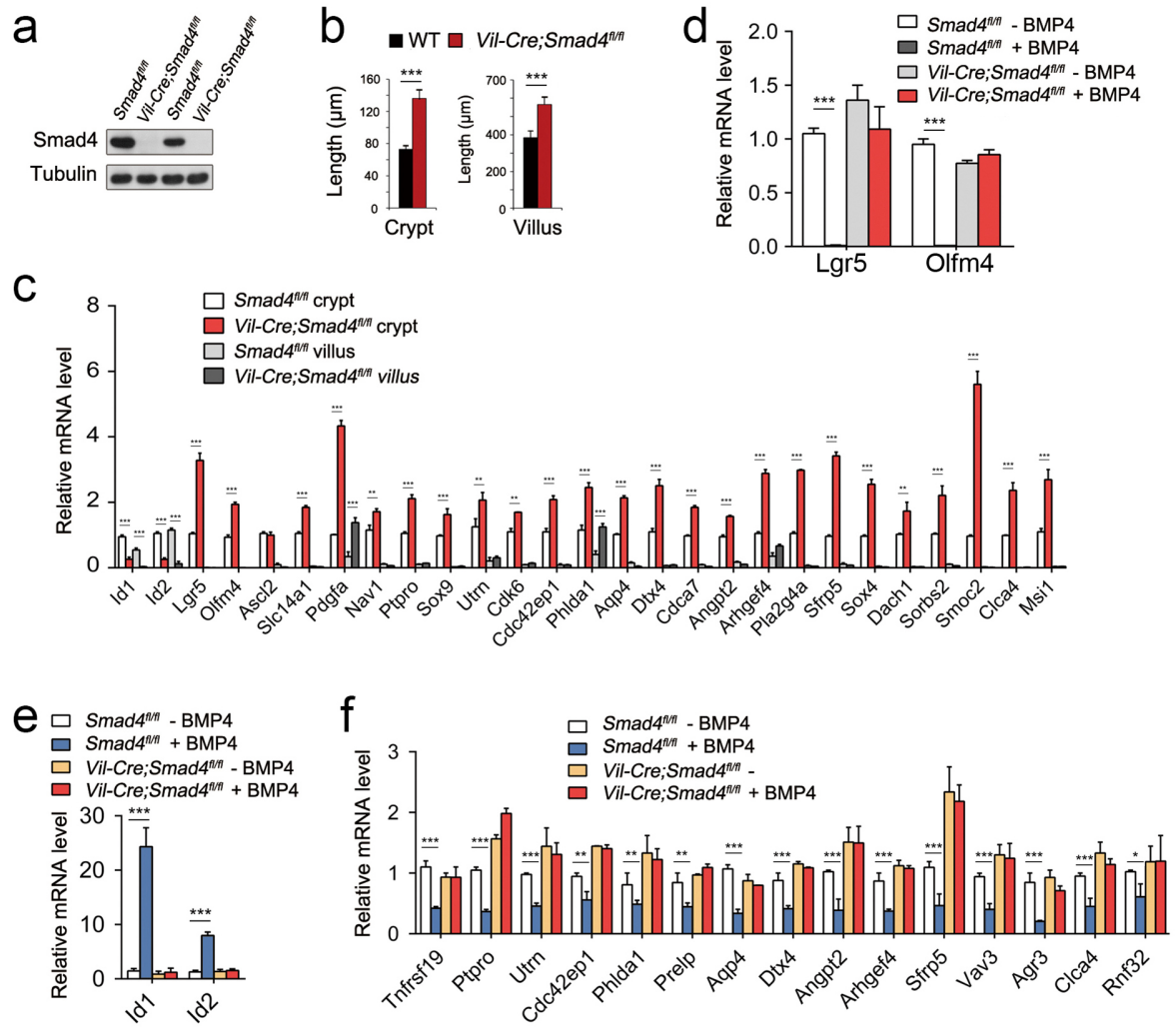
Supplementary Figure 5 Basal BMP activity constrains $Lgr5^{+}$ stem cell expansion in the organoids cultured in the normal ENR medium.

(a) Increase in Noggin concentration (100 ng ml^{-1} or 400 ng ml^{-1}) significantly blocked basal BMP activity, as indicated by downregulation of *Id1* and upregulation of *Lgr5* in organoids. Data represent mean \pm s.e.m of three independent experiments. $***P < 0.001$ by Student's *t*-tests. (b) Quantitative RT-PCR analysis of *Id1* and signature gene expression in organoids cultured for 5 days in the presence of Noggin. Data represent mean \pm s.e.m of three independent experiments. $***P < 0.001$ by Student's *t*-tests. (c) Confocal and bright-field images of Lgr5-EGFP (for $Lgr5^{+}$ stem cells) in organoids cultured for 7 days with different amount of Noggin. Images are representative of three independent experiments. (d) Flow cytometry analysis of Lgr5-EGFP expression 5 days or 7 days after cultured with different amount of Noggin. Images are representative of three independent experiments. Scale bar, $50 \mu\text{m}$.



Supplementary Figure 6 Smad4 binds to the promoter or enhancer regions of BMP target genes in *Lgr5*⁺ stem cells.

(a) Heatmap of Smad4 bound regions relative to transcription start sites. (b) GO analysis of BMP-upregulated genes or -downregulated genes that were bound by Smad4. (c) Visualization of the Smad4 binding site and the TCF4 binding site in the first intron of *Lgr5*. The TCF4 binding site was identified based on TCF4 ChIP-seq in intestinal crypts (GEO: GSE65322). (d) β-catenin ChIP assays in *Lgr5*⁺ stem cells with or without 4 hour BMP4 treatment. Data represent mean ± s.e.m of three independent experiments. (e) Smad4 ChIP signals at the promoter or enhancer regions of representative cell cycle arrest genes.



Supplementary Figure 7 Deletion of Smad4 abolishes BMP response in organoids.

(a) Immunoblots of small intestine epithelium from 1-month old *Smad4^{fl/fl}* and *Vil-Cre;Smad4^{fl/fl}* mice. Images are representative of n=3 mice per genotype. (b) Quantification of crypt length and villus length in 1-month old *Smad4^{fl/fl}* and *Vil-Cre;Smad4^{fl/fl}* mice. Data represent mean \pm s.e.m of n=5 mice per genotype. *** $P < 0.001$ by Student's *t*-tests. (c) Quantitative RT-PCR analysis of *Id1*, *Id2* and BMP-regulated signature gene expression in crypts or villus from the mice with indicated genotypes. Data represent mean \pm s.e.m of n=5 mice per genotype. ** $P < 0.01$, *** $P < 0.001$ by Student's *t*-tests. (d) Quantitative RT-PCR analysis of *Lgr5*, *Olfm4* and *Ascl2* expression in the organoids treated with or without BMP4 for 36 hours. Data represent mean \pm s.e.m of three independent experiments. *** $P < 0.001$ by Student's *t*-tests. (e,f) Quantitative RT-PCR analysis of gene expression in the organoids derived from the mice with indicated genotypes after treated with BMP4 for 4 hours. Data represent mean \pm s.e.m of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's *t*-tests. Scale bars, 50 μ m.

Fig. 4c

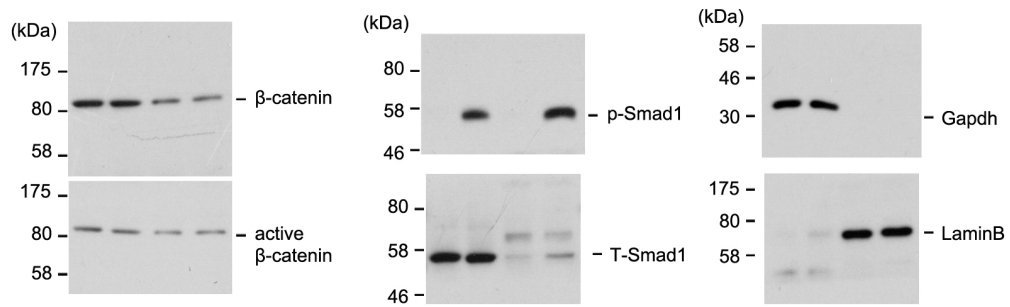
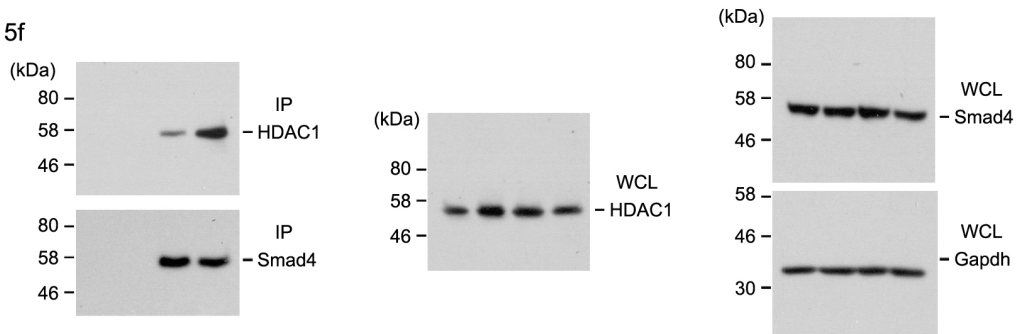
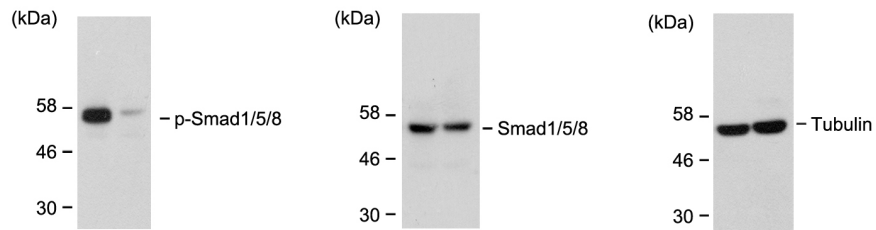


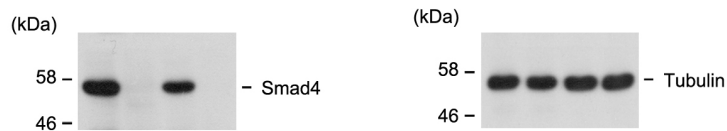
Fig. 5f



Supplementary Fig. 1c



Supplementary Fig. 7a



Supplementary Figure 8 Uncropped scans of western blots included in main figures.